CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER:

761178Orig1s000

NON-CLINICAL REVIEW(S)

MEMORANDUM

DEPARTMENT OF HEALTH & HUMAN SERVICES
Public Health Service
Food and Drug Administration
Center for Drug Evaluation and Research

Date: June 7, 2021

From: Lois M. Freed, Ph.D.
Director, Division of Pharmacology/Toxicology-Neuroscience
Office of Neuroscience

Subject: BLA 761178 (Aduhelm, aducanumab, 12F6A)

BLA 761178 was submitted on July 7, 2020, for marketing authorization of aducanumab to "…delay clinical decline in patients with Alzheimer's disease." The nonclinical studies to support clinical development and the BLA consisted of primary and secondary pharmacology, PK/TK, general toxicology, and reproductive and developmental toxicology studies. These studies have been reviewed by Dr. Hawver (Pharmacology/Toxicology BLA Review and Evaluation, David B. Hawver, Ph.D., May 14, 2021), who has concluded the data are adequate to support approval of aducanumab for the proposed indication.

This memo provides a brief summary of the nonclinical data, focusing on those important for labeling.

Aducanumab is a fully human IgG1 monoclonal antibody intended to bind aggregated soluble and insoluble forms of human amyloid beta (Aβ) to lower amyloid plaques in brain. The sponsor proposes that this mechanism will result in a delay in the cognitive decline in Alzheimer's disease patients.

The primary pharmacology of aducanumab and identification of its epitope was tested in in vitro assays and in ex vivo assays in brain tissue from AD patients and Tg2576 mice. In vivo studies in the Tg2576 mouse model were conducted using the murine analog, ch12F6A, a chimeric anti-Aβ monoclonal antibody.

Sequence homology between human and monkey Aβ42 is 100% and between human and rodent (mouse and rat) Aβ is 92.9%. However, the intended target of aducanumab is aggregated forms of Aβ peptide. Neither rat nor monkey is a particularly relevant species for general toxicity because of the lack of aggregated amyloid in rodent or non-aged monkey. Therefore, the chronic (6-

month) toxicity of the murine analog (^{ch}12F6A) was tested in the Tg2576 mouse; shorter-duration toxicity studies were conducted in monkey (4-week) and Tg2576 mouse (13-week). The monkey study confirmed the lack of toxicity due to any unintended binding.

In the 4-week study in monkey (2.6-4.9 yrs), aducanumab (0, 10, 100, or 300 mg/kg IV QW) resulted in no drug-related effects.

In Tg2576 mouse, ^{ch}12F6A was administered for 13 weeks (0, 10, 70, or 500 mg/kg) or 6 months (0, 10, 40, or 250 mg/kg) weekly by intravenous injection; in each study, a separate group received aducanumab (500 or 250 mg/kg, respectively) according to the same regimen. In the 13-week study, dosing was initiated at ~79-80 weeks of age, at which time plaques in multiple brain regions are expected. No adverse effects were observed with aducanumab. ^{ch}12F6A resulted in an increase in histopathological changes in brain vasculature (inflammation and hemorrhage) at the mid and high doses, and an increase in vascular thrombosis at the high dose. In addition, the presence of amyloid in brain (plaques) and vasculature was confirmed in all but one animal. At the end of the 6-week recovery period, the incidence of vascular effects was similar among groups.

In the 6-month study in Tg2576 mouse, dosing was initiated at 16-17 months (~70-74 weeks) of age. No adverse effects were observed with aducanumab. ^{ch}12F6A resulted in minimal to mild histopathological changes in brain vasculature. According to the sponsor, there was a “slight increase” in the incidence or severity of brain vascular inflammation or thickening at the mid and high doses. At the end of the 6-week recovery period, the incidence of vascular effects was similar among groups.

Potential effects on reproductive and developmental parameters of aducanumab (0, 100, 300, or 1000 mg/kg/week IV) were assessed in a standard battery (fertility and early embryonic development, embryofetal development, and pre- and postnatal development) of studies in rat. No adverse effects on reproductive or developmental parameters were observed.

Genetic toxicology studies were not conducted as they are not considered necessary for biologics products.

Carcinogenicity studies were also not conducted, as agreed to by the division (June 14, 2016).

Conclusion and Recommendation

From a nonclinical standpoint, the nonclinical studies conducted for aducanumab are adequate to support approval of the BLA for the intended patient population.

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/s/

LOIS M FREED
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DEPARTMENT OF HEALTH AND HUMAN SERVICES
PUBLIC HEALTH SERVICE
FOOD AND DRUG ADMINISTRATION
CENTER FOR DRUG EVALUATION AND RESEARCH

PHARMACOLOGY/TOXICOLOGY BLA REVIEW AND EVALUATION

Application number:	761178
Supporting document:	1, 65
Applicant's letter date:	February 20, 2020; January 27, 2021
CDER stamp date:	February 20, 2020; January 27, 2021
Product:	ADUHELM (Aducanumab)
Indication:	To delay clinical decline in patients with Alzheimer's disease
Applicant:	Biogen Inc.
Division:	Pharmacology/Toxicology-Neuroscience
Reviewer:	David B. Hawver, Ph.D.
Supervisor:	Lois M. Freed, Ph.D.
Project Manager:	Emilios A. Papanastasiou

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1 Executive Summary

1.1 Introduction

Aducanumab-avwa is a human immunoglobulin gamma 1 (IgG1) monoclonal antibody that binds to aggregated soluble and insoluble forms of amyloid beta (Aβ), resulting in the removal of amyloid plaques from the brain. The proposed indication is “to delay clinical decline in patients with Alzheimer’s disease” in whom the presence of amyloid beta pathology has been confirmed.

1.2 Brief Discussion of Nonclinical Findings

Pharmacology studies demonstrated that aducanumab (and/or a chimeric murine version, ^{ch}12F6A) specifically binds to aggregated forms of human Aβ peptides (plaques, fibrils, protofibrils, and oligomers), resulting in significant reductions in insoluble Aβ and amyloid plaques in the brain of aged Tg2576 mice, which overexpress a mutant form of Amyloid Precursor Protein that causes early onset Alzheimer’s disease in humans. The toxicology of aducanumab was adequately assessed in general toxicity studies in monkey and Tg2576 mouse. In the pivotal repeated-dose study in monkey, administration of aducanumab (0, 10, 100, or 300 mg/kg/week IV) for 4 weeks resulted in no adverse effects. Aducanumab plasma exposure (AUC) after 4 doses at 300 mg/kg/week was ~35-fold greater than that expected in humans at steady-state at the maximum proposed clinical dose (10 mg/kg IV every 4 weeks) after adjusting for differences in the dosing interval.

In a pivotal 13-week repeated-dose study in Tg2576 mouse, IV administration of ^{ch}12F6A (0, 10, 70, or 500 mg/kg/week) resulted in increased incidence and/or severity of meningeal and/or cerebral vascular inflammation, thrombosis, and/or hemorrhage at the mid and high doses consistent with slight exacerbation of the cerebral amyloid angiopathy that increases with aging in this mouse model. The no-observed-adverse-effect-level (NOAEL) was 10 mg/kg/week. The vascular effects were similar to the drug-related increases in Amyloid-Related Imaging Abnormalities (ARIA; vascular edema and microhemorrhages) reported in clinical studies of aducanumab.

No clear drug-related adverse effects were observed in a pivotal 6-month study of ^{ch}12F6A (0, 10, 40, or 250 mg/kg/week IV) in Tg2576 mouse. The inconsistent results between the 13-week and the 26-week studies may be related to the difference in the age of the animals at initiation of dosing (79-80 and 69-73 weeks old, respectively).

There were no adverse effects on fertility and early embryonic development, embryofetal development, or pre- and postnatal development in studies of aducanumab (0, 100, 300, and 1000 mg/kg/week IV) conducted in rat.

1.3 Recommendations

1.3.1 Approvability

The nonclinical data submitted adequately support the approval of aducanumab-avwa for the treatment of patients with Alzheimer's disease.

1.3.2 Additional Nonclinical Recommendations

None

1.3.3 Labeling

The sponsor's proposed labeling for the nonclinical sections should be revised as specified below (suggested deletions are crossed out; additions are underlined and bolded):

8.1 Pregnancy

Risk Summary

There are no adequate data on (b) (4) in pregnant women. In the U.S. general population, the estimated background risk of major birth defects and miscarriage in clinically recognized pregnancies is 2 to 4% and 15 to 20%, respectively. The background risk of major birth defects and miscarriage for the indicated population is unknown.

Data

Animal Data

(b) (4)

8.2 Lactation

Risk Summary

There are no data on the presence of aducanumab-avwa in human milk, the effects on the breastfed infant, or the effects of the drug on milk production. The developmental and health benefits of breastfeeding should be considered along with the mother's clinical need for ADUHELM and any potential adverse effects on the breastfed infant from ADUHELM or from the underlying maternal condition.

8.4 Pediatric Use

Safety and effectiveness in pediatric patients have not been established.

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12 Clinical Pharmacology

12.1 Mechanism of Action

Aducanumab-avwa is a human, immunoglobulin gamma 1 (IgG1) monoclonal antibody (mAb) (b) (4) aggregated soluble and insoluble forms of amyloid beta, (b) (4). Aducanumab-avwa (b) (4) **reduces** amyloid plaques (b) (4)

……

13 Nonclinical Toxicology

13.1 Carcinogenesis, Mutagenesis, Impairment of Fertility

Carcinogenesis

(b) (4)

Mutagenesis

Genotoxicity studies have not been conducted.

Impairment of Fertility

(b) (4)

(b) (4)

2 Drug Information

2.1 Drug

Nonproprietary Name
aducanumab-avwa

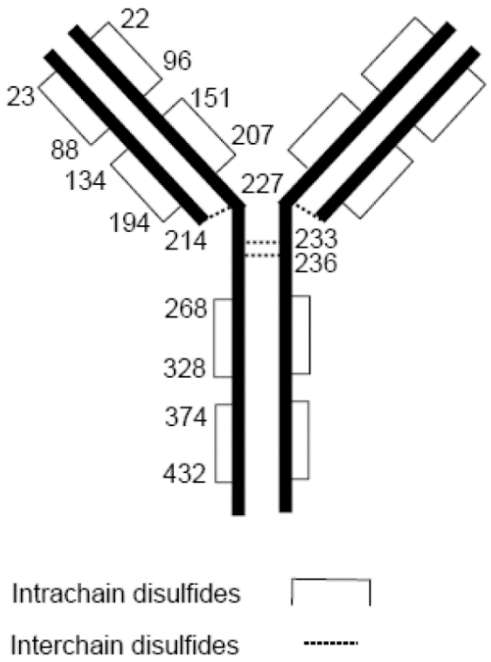
Proprietary Name
ADUHELM

Code Name
BIIB037, 12F6A, wt hu12F6A

CAS Number
1384260-65-4

Molecular Weight
146 kDa (peptide sequences only)

Biochemical Description
Aducanumab is a recombinant human IgG1 monoclonal antibody targeting soluble and insoluble forms of the human Aβ peptide. Two heavy and two kappa light chains are connected by inter-chain disulfide bonds. Carbohydrate moieties are linked to asparagine residue 304 on the heavy chains. The structure of the fully assembled protein is shown in the figure below:



(page 7, section 2.3.S, Drug Substance)

Pharmacologic Class
Aducanumab-avwa is a human IgG1 monoclonal antibody targeting aggregated forms of human Aβ.

2.2 Relevant INDs, NDAs, BLAs, and DMFs

IND 106230 aducanumab for the treatment of Alzheimer's disease

2.3 Drug Formulation

Aducanumab is formulated as a sterile solution for IV infusion in a single-dose vial. The concentrations of aducanumab and other components of the solution are listed in Table 2 below:

Table 2: Formulation History of Drug Product During Clinical Development

	Formulation: Process A and Process B	Formulation: Process C (Commercial Formulation)
Formulation	(b) (4)	100 mg/mL aducanumab 16.2 mM L-histidine hydrochloride, monohydrate 3.8 mM L-histidine 150 mM L-arginine hydrochloride 10 mM L-methionine 0.05% (w/v) polysorbate 80 pH 5.5
Clinical Usage	Phase 1 studies Phase 3 studies	Phase 2 studies Phase 3 studies

(page 8, section 2.3.P, Drug Product; Process A was used for nonclinical studies)

2.4 Comments on Novel Excipients

No novel excipients are present in the drug product.

2.5 Comments on Impurities/Degradants of Concern

No impurities or degradants of concern were identified.

2.6 Proposed Clinical Population and Dosing Regimen

The proposed indication is for the treatment of patients with Alzheimer's disease (b) (4) The proposed dosing regimen is administration by IV infusion over approximately one hour every four weeks, titrating upward from 1 mg/kg (infusions 1 and 2) to 3 mg/kg (infusions 3 and 4) to 6 mg/kg (infusions 5 and 6) to 10 mg/kg thereafter.

2.7 Regulatory Background

During a Pre-IND meeting on October 13, 2009, the sponsor was informed that "the use of the Tg2576 mouse as the sole animal species for toxicity testing would need to be adequately justified" using quantitative data on the binding of aducanumab to fibrillar and soluble forms of Aβ; that a toxicity study in the nonhuman primate should be conducted using the clinical candidate; that the proposed use of a murine chimeric IgG2a surrogate antibody for evaluation of the potential for aducanumab to cause microhemorrhage and vascular edema in Tg2576 mice appeared to be appropriate; and that the failure to detect exacerbation of cerebral microhemorrhage in Tg2576 mice following administration of the positive control antibody (3D6) would need to be addressed (Pre-IND 106230 Meeting Minutes, November 19, 2009).

In the May Proceed Letter (May 31, 2011), the sponsor was asked "to provide a detailed description of how the distinction between specific and nonspecific labeling of BIIB037 was made, including the exact criteria used for defining specific binding, as well as

justification for the use of this methodology" in the tissue cross-reactivity study. The justification provided in the submission of September 19, 2011, was adequate.

In response to a request for comments (October 24, 2011) on the protocol for a planned 26-week toxicity study in Tg2576 mice and on the overall nonclinical development plan, specific comments were provided (November 29, 2011) on the protocol, and the sponsor was informed that the 6-month study would be sufficient to characterize the chronic toxicity of aducanumab and that reproductive and developmental toxicology studies may be needed if the indication is modified to include a younger patient population.

In an End-of-Phase 2 Meeting (see January 16, 2015, Minutes), the sponsor was informed that the completed general toxicology studies appeared to support a BLA for aducanumab, that a request (with detailed rationale and supportive data) to not conduct carcinogenicity studies should be submitted to the IND, and, as recommended by the Controlled Substances Staff, clinical trials should include monitoring for adverse events that may indicate a potential for abuse of aducanumab.

The Division and the Executive Carcinogenicity Assessment Committee agreed (September 11, 2015) with the sponsor's justification for not conducting carcinogenicity studies of aducanumab.

3 Studies Submitted

3.1 Studies Reviewed

Pharmacology
In Vitro Functional Characterization and Selectivity of Anti-Aβ Monoclonal Antibody 12F6A

Binding Selectivity and Epitope of Aducanumab

Efficacy of murine chimeric anti-Aβ monoclonal antibodies ch12F6A and ch12F6A Agly in the Tg2576 transgenic mouse model

Efficacy of murine chimeric anti-Aβ monoclonal antibody ch12F6A in the Tg2576 transgenic mouse model

Brain Penetration and Target Engagement of Anti-Aβ Monoclonal Antibody BIIB037 (12F6A) in Tg2576 transgenic mice

Immunotherapy with aducanumab restores calcium homeostasis in Tg2576 mice

Pharmacokinetics

Analytical Methods, Qualification, and Validation Studies

The Determination of the Concentration of BIIB037 (human BART) in Mouse Plasma (K_2EDTA) by Quantitative ELISA

Validation of a Solution Enzyme-linked Immunosorbent Assay (ELISA) for the Detection and Confirmation of Anti-BIIB037 Antibodies in Mouse Plasma (K_2EDTA)

Detection of Anti-BIIB037 Antibodies in Support of Preclinical Study P037-09-02 (BIIB037: A 13-Week Toxicity Study Administered by Weekly Slow Bolus Injection to Tg2576 Mice With a 6-Week Recovery Period)

Development and Qualification of an ELISA to Determine the Concentration of Chimeric 12F6A (BART) in C57BL/6 and Transgenic (Tg2576) Mouse Plasma

The Determination of the Concentration of BIIB037 Murine Chimeric (Chimeric BART; 12F6A IgG2a) in Mouse Plasma (K_2EDTA) by Quantitative ELISA

Validation for the Detection of Anti-chimeric 12F6A WT BART Antibodies in Mouse Plasma (K_2EDTA) by Solution ELISA

Detection of Anti-Chimeric BIIB037 Antibodies in Support of Preclinical Study P037-09-02 (BIIB037: A 13-Week Toxicity Study Administered by Weekly Slow Bolus Injection to Tg2576 Mice With a 6-Week Recovery Period)

Validation of an Enzyme Linked Immunosorbent Assay (ELISA) Method for the Determination of the Concentration of BIIB037 in Cynomolgus Monkey Serum

The Determination of the Concentration of BIIB037 (12F6A) in Cynomolgus Monkey Plasma K_2EDTA by Quantitative ELISA

Validation for the Detection of Anti-BIIB037 Antibodies in Cyno Plasma (K_2EDTA) by Solution ELISA

Validation of an Enzyme-Linked Immunosorbent Assay (ELISA) Method for the Determination of the Concentration of BIIB037 in Rat Serum

Absorption

Pharmacokinetics (PK) of Chimeric 12F6A IgG2a (ch12F6A) in Tg2576 and Aged Wild-Type Mice Following a Single Intravenous (IV), Intraperitoneal (IP) or Subcutaneous (SC) Injection

Repeat-Dose Toxicity

BIIB037: A 4 Dose, Once Weekly Subcutaneous Injection Tolerability and Toxicokinetic Study in Cynomolgus Monkeys

BIIB037: A 4-Week Tolerability and Toxicokinetic Study when Administered by Intravenous and Subcutaneous Injection to Cynomolgus Monkeys

BIIB037: 4 Week Toxicity Study of BIIB037 When Administered By Slow Intravenous Bolus Injection To Cynomolgus Monkeys With An 8-Week Recovery Period

BIIB037: A 13-Week Toxicity Study Administered by Weekly Slow Bolus Injection to Tg2576 Mice With a 6-Week Recovery Period

BIIB037: A 6 Month Study of BIIB037 by Weekly Intravenous Injection in the Tg2576 Mouse with a 6 Week Recovery Period

Reproductive and Developmental Toxicology
BIIB037: A Once Weekly Intravenous Injection Fertility and Early Embryonic Development Study with a Toxicokinetic Phase in Male and Female Sprague Dawley Rats

BIIB037: An Intravenous Injection Embryo/Fetal Development Study with a Toxicokinetic Phase in Female Sprague Dawley Rats

BIIB037: An Intravenous Injection Pre- and Postnatal Development Study, Including Maternal Function, in Sprague Dawley Rats

Other
Tissue Cross-Reactivity of BIIB037 with Human, Mouse and Cynomolgus Monkey Tissues *Ex Vivo*

In Vitro Evaluation of the Influence of BIIB037 on Human Whole Blood Hemolysis and Plasma Flocculation

In Vitro Evaluation of the Influence of BIIB37 on Human Whole Blood Hemolysis and Plasma Flocculation

3.2 Studies Not Reviewed

None

3.3 Previous Reviews Referenced

IND 106230 Aducanumab for Alzheimer's Disease

- Pharmacology/Toxicology 30-Day Safety Memorandum, Barbara Wilcox, Ph.D., February 3, 2012
- Pharmacology and Toxicology Review of rationale for not conducting carcinogenicity studies, Barbara Wilcox, Ph.D., June 23, 2020

4 Pharmacology

4.1 Primary Pharmacology

In Vitro Functional Characterization and Selectivity of Anti-Aβ Monoclonal Antibody 12F6A

(Biogen Idec Study Rsch-2010-029; Final Report, January 7, 2011; non-GLP)

In immunoprecipitation and immunoblotting experiments with different forms of human Aβ42, aducanumab (12F6A) showed binding to high molecular weight, aggregated, fibrillar Aβ42, little or no binding to monomeric Aβ42, and no binding to soluble Aβ42 oligomers.

In ELISA binding studies, aducanumab showed high affinity for immobilized fibrils of Aβ42 or Aβ40 (EC_{50} ~0.1 nM) at all concentrations evaluated ([fibril] = 0.1, 1, or 10 µg/mL); similar affinity was observed with IgG2a/kappa ^{ch}12F6A, a mouse chimeric variant of aducanumab. Binding affinity of aducanumab for immobilized Aβ42 or Aβ40 monomers was concentration dependent: EC_{50} = 20, 0.16, and 0.09 nM, at [monomer] = 0.1, 1, or 10 µg/mL, respectively. In additional studies, aducanumab showed selectivity for immobilized human Aβ42 monomers compared to mouse Aβ42 monomers (EC_{50} = 0.5 and ~20 nM, respectively, at 2 µg/mL).

In inverted ELISA binding studies, there was minimal binding to soluble biotinylated Aβ40 with aducanumab and ch12F6A (EC_{50} > 1000 nM). Biotinylated Aβ40 was used because Aβ42 is poorly soluble in aqueous solution.

In vitro binding of aducanumab to human Fcγ receptors (IC_{50} = 7 and 0.1 µg/mL for CD16 and CD64, respectively) was shown to be similar to that for 5C8, a reference human IgG1 control antibody (IC_{50} = 3 and 0.08 µg/mL for CD16 and CD64, respectively).

In vitro binding of aducanumab to human complement C1q was similar to that for control antibody 5C8 (EC_{50} ~2 µg/mL).

In immunocytochemistry studies, aducanumab demonstrated stronger binding to amyloid plaques in frozen compared to formalin-fixed brain sections from a patient with Alzheimer's disease (AD).

Binding Selectivity and Epitope of Aducanumab

(Biogen Idec Study Rsch-2018-035v2; Final Report, January 2019; non-GLP)

In ELISA binding studies, ^{ch}12F6A showed selectivity for immobilized fibrillar Aβ42 compared to monomeric soluble Aβ40 (EC_{50} = 0.2 nM and >1 µM, respectively).

In a competition ELISA, ^{ch}12F6A bound to immobilized oligomeric Aβ42 despite the presence of soluble monomeric Aβ40 (IC_{50} >1 µM).

In an ELISA using immobilized streptavidin (which binds to biotin) and soluble biotinylated monomeric Aβ40, ^{ch}12F6A Fab fragments showed binding to the monomeric Aβ40 (EC_{50} = 540 nM).

In an ELISA using immobilized anti-mouse IgG in the presence of soluble biotinylated multi-antigen peptides (with two or four copies of $Aβ_{1-15}$), the tetrameric Aβ peptide showed binding to ^{ch}12F6A, but the dimeric peptide did not (EC_{50} = 6.81 nM and >400 nM, respectively).

In immunoprecipitation studies, dot blots showed ^{ch}12F6A immunoprecipitated Aβ42 soluble oligomers and insoluble fibrils, but not soluble monomers.

In ex vivo immunocytochemistry studies, strong staining of amyloid plaques in brain sections from a patient with AD and from a 22-month-old Tg2576 transgenic mouse was observed with ^{ch}12F6A and aducanumab, respectively.

Using surface plasmon resonance, the K_D for binding of Fab fragments of ^{ch}12F6A to biotinylated monomeric Aβ40 was ~9 µM.

Using solution-based methods, ^{ch}12F6A showed binding to monomeric $Aβ_{1-16}$ by microscale thermophoresis and for monomeric $Aβ_{1-28}$ by isothermal titration calorimetry (K_D = 1.8 µM and 5.9 µM, respectively).

Using negative-stain electron microscopy, gold-conjugated ^{ch}12F6A showed significantly greater binding to Aβ40 and Aβ42 fibrils compared to gold-conjugated 3D6 ($p<0.0001$, two-way ANOVA). There was no specific binding to tau fibrils.

Epitope mapping using ELISA with truncated Aβ peptides demonstrated the importance of Aβ residues 3 to 9 in the binding of aducanumab. Further studies using peptide array and alanine substitution scanning methods indicated that aducanumab binds to Aβ residues 3 to 7, with amino acids Phe4 and His6 forming the core epitope.

Crystallographic analyses of the aducanumab Fab fragment-$Aβ_{1-11}$ peptide complex showed that the key interactions were between the complementarity-determining regions of aducanumab (heavy chain CDRs H2 and H3, and light chain CDRs L3 and L1) and residues Phe4, His6, Glu3, and Arg5 of the $Aβ_{1-11}$ peptide. Twelve residues in the aducanumab CDR sequences were within 4 Å of the Aβ peptide.

Efficacy of murine chimeric anti-Aβ monoclonal antibodies ch12F6A and ch12F6A Agly in the Tg2576 transgenic mouse model
(Biogen Idec Study Rsch-2011-006; Final Report, February 14, 2011; non-GLP)

Tg2576 transgenic mice (12-14/group at termination) were administered 3 mg/kg ^{ch}12F6A, 3 mg/kg aglycosylated ^{ch}12F6A (^{ch}12F6A Agly), 3 mg/kg 3D6, or vehicle (PBS) via weekly IP injections for 25 weeks, from age 9.5 months to 15.5 months. Reductions were observed in cerebral formic-acid extracted insoluble Aβ40 and Aβ42 (52% and 55%, respectively), Congo-Red positive Aβ plaque area (44% in cortex) and number (67% in cortex, 60% in hippocampus), and Thioflavin-S positive Aβ plaque area (43% in cortex) and number (27% in cortex, 38% in hippocampus) in ^{ch}12F6A-dosed animals. Analysis of the CA1-3 subfields and the dentate gyrus, omitting the subicular complex, showed significant reductions in Thioflavin-S positive plaque area (42%) and number (41%). No significant effects on brain Aβ were seen in ^{ch}12F6A Agly-dosed animals, suggesting that the Fc effector function previously demonstrated to be reduced in the aglycosylated variant is important to the ability of ^{ch}12F6A to reduce brain amyloid. There were significant improvements in memory performance in a contextual fear conditioning behavior assessment after 6 months of dosing with ^{ch}12F6A or ^{ch}12F6A Agly. No significant effects on brain Aβ or behavioral performance were seen in 3D6-dosed animals.

Efficacy of murine chimeric anti-Aβ monoclonal antibody ch12F6A in the Tg2576 transgenic mouse model
(Biogen Idec Study Rsch-2011-007; Final Report, February 16, 2011; non-GLP)

In Study BART-09-B06, Tg2576 mice were administered ^{ch}12F6A (0 [PBS], 0.3, 1, 3, or 10 mg/kg via weekly IP injections for 24 weeks, from age 36 weeks to 60 weeks, and terminated 1, 4, or 7 days after the final dose (N=19-24, 3-4, and 4-9/group, respectively). Study BART-09-N07 used a different colony of Tg2576 mice given 0, 3, 10, or 30 mg/kg IP QW for 24 weeks, starting at age 38-42 weeks, terminated 1 or 7 days after the final dose (N=13-19 and 5/group, respectively). Data from the two studies were combined for analyses. Median plasma drug concentrations were 0.0450, 0.0500, 4.950, 14.85, 49.20, and 144.0 µg/mL one day after the final doses of 0, 1, 3, 10, and 30 mg/kg, respectively. Median brain drug concentrations were 27.00, 27.00, 27.00, 37.15, 228.7, and 1377 ng/g one day after the final doses of 0, 1, 3, 10, and 30 mg/kg, respectively. Statistically significant reductions were observed in brain concentrations of Aβ40 (~30-40%) and Aβ42 (~40-50%) in the soluble diethylamine and the insoluble guanidine fractions from animals dosed at 3 (Aβ42 only), 10, or 30 mg/kg. Statistically significant reductions also occurred in the total Aβ plaque area as determined by staining with 6E10 anti-Aβ antibody or Thioflavin-S in cortex (~50%) and hippocampus (~60-70%) from animals dosed at 10 or 30 mg/kg; animals given 3 mg/kg showed significant reductions in Thioflavin-S staining in cortex (40%).

Qualitative analyses of brain sections immunostained with 6E10 (for Aβ plaques) and Iba-1 (for activated microglia) revealed sharply defined, small, compact, dense plaques surrounded by amoeboid activated microglia in ^{ch}12F6A-dosed animals (3 mg/kg was

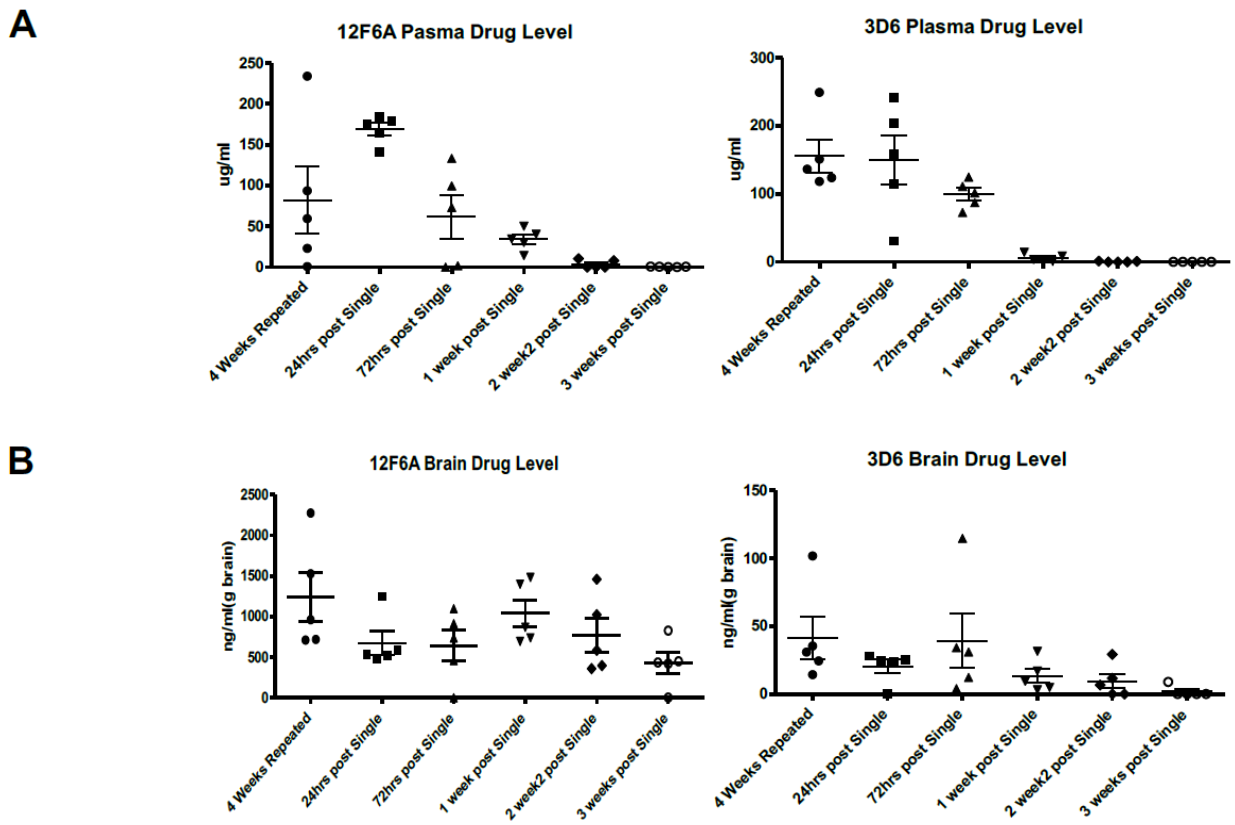
the only dose evaluated). In contrast, animals given PBS showed irregular, fuzzy-edged plaques rarely associated with activated microglia.

Brain Penetration and Target Engagement of Anti-Aβ Monoclonal Antibody BIIB037 (12F6A) in Tg2576 transgenic mice
(Biogen Idec Study Rsch-2018-034; Final Report, May 5, 2018)

Male and female Tg2576 mice (17-18 months old, N=3F and 2M/group/time point) were administered a single dose of 30 mg/kg aducanumab (12.05 mg/mL) or 3D6 antibody (4.25 mg/mL) via IP injection and terminated at 24 hours, 72 hours, 1 week, 2 weeks, or 3 weeks after dosing. Additional groups were administered aducanumab or 3D6 at 30 mg/kg IP weekly for 4 doses and were terminated 24 hours after the final dose.

Plasma and brain aducanumab and 3D6 following a single dose are shown in Figure 1 below. Aducanumab exposure in brain ($AUC_{0\text{-}504\ hr}$) was 1.3% of plasma exposure.

Figure 1. Plasma and Brain Drug levels in study BART-11-N09



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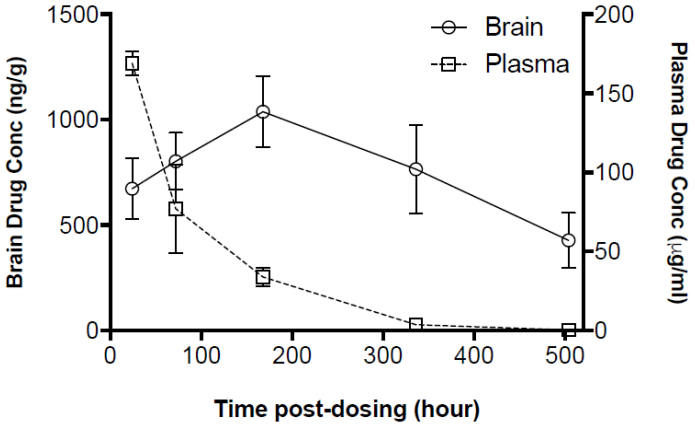

Plasma (A and C) and brain (B and C) drug levels measured at different timepoints following single i.p. administration of either BIIB037 or 3D6. n=5 per timepoint; mean ± S.E.M.

(pages 11-12 of Study Report)

Plasma Aβ38, Aβ40, and Aβ42 increased substantially after administration of 3D6, but did not increase after administration of aducanumab, suggesting that aducanumab does not bind or stabilize Aβ monomers in plasma.

Immunohistochemical analyses of brain sections demonstrated binding of aducanumab to most of the parenchymal amyloid plaques and some of the vascular amyloid deposits that were stained with anti-Aβ antibody 6E10 in adjacent sections.

Immunotherapy with aducanumab restores calcium homeostasis in Tg2576 mice

(Biogen Idec Study Rsch-2018-036; Final Report, December 5, 2018)

Acute Study

Following craniotomy and durotomy, ^{ch}12F6A or P1.17 (a negative control mouse IgG2 antibody) was topically applied to the brain surface of 22-month-old Tg2576 mice (N=3-4/group) at 0.4-1 mg/mL for 20 minutes; the application site was covered with a glass coverslip and animals were administered 10 mg/kg IP methoxy-XO4 to visualize amyloid plaques. Effects of antibodies on clearance of existing amyloid plaques and deposition of new plaques were determined by assessing plaque area and number on the day of topical application and 3 weeks later, using two-photon imaging microscopy.

Application of ^{ch}12F6A resulted in disappearance of 48% of existing plaques and a 27% reduction in total plaque area. In contrast, P1.17-dosed animals showed disappearance of 14% of existing plaques and a 33% increase in plaque area. There were no significant effects on the deposition of new plaques or the pattern or extent of cerebral amyloid angiopathy.

Chronic Study

The somatosensory cortex of 18-month-old Tg2576 mice was injected with AAV8-YC3.6 (a viral construct design to express linked cyan and yellow fluorescent proteins in

infected neurons, allowing measurement of calcium concentrations) and an 8 mm cranial window was made to allow serial brain imaging, using 10 mg/kg IP methoxy-XO4 to visualize amyloid plaques. Mice were administered 10 mg/kg IP ^{ch}12F6A (N=5) or P1.17 (N=3) weekly for 6 months, and were imaged at baseline, 2 weeks after initiation of dosing, and monthly throughout the end of the dosing period.

Fluorescent Cy3-tagged ^{ch}12F6A injected IP colocalized with methoxy-XO4 positive amyloid plaques, confirming engagement of the desired target in the brain. There were no significant effects on plaque clearance, total area, size, or number. Baseline measurements of calcium levels in 18-month-old Tg2576 mouse cortical neurites imaged through the cranial windows showed 18.5% of neurites exhibited calcium overload, compared to 2.2% in 18-month-old WT mice. After 2 weeks of treatment, the % of neurites with calcium overload was reduced (8% vs. 21% in controls). After 6 months, 0% of cortical neurites showed elevated calcium in ^{ch}12F6A-dosed Tg2576 mice (N=139 neurites in 5 mice), compared to 28% in P1.17-dosed animals (N=135 neurites in 3 mice). Dosing with ^{ch}12F6A also prevented the increase in percentage of cortical neuronal cell bodies exhibiting calcium overload observed in P1.17-dosed Tg2576 mice as they aged.

Nonclinical Information Submitted January 27, 2021, in Response to FDA Request for Information Regarding Potential for Acute Treatment Effects of Aducanumab

The sponsor provided published references in support of the idea that aducanumab may interfere with Aβ oligomer-related synaptic dysfunction to (theoretically) provide a short-term cognitive benefit, in addition to any long-term effects related to clearance of amyloid plaques from the brain. The evidence was provided that: 1) Aβ oligomers associate with synapses and can “induce aberrations in synapse composition, shape, and density near amyloid plaques”; 2) Aβ oligomers can disrupt synaptic plasticity and memory via inhibition of hippocampal long-term potentiation (LTP), facilitation of long-term depression (LTD), and alteration of the excitatory/inhibitory balance; and 3) antibodies targeting Aβ oligomers can reduce neuroinflammation prevent adverse effects on neuronal structure and function.

<u>Effects of Aβ oligomers on synaptic structure</u>
Using an APP/PS1 mouse model, Koffie et al. (2009) showed that brain volumes immediately surrounding amyloid plaques had much higher concentrations of Aβ oligomers and 60% lower density of excitatory synapses compared to similar volumes away from plaques, and that postsynaptic densities (PSDs) were significantly smaller when they were in contact with microdeposits of Aβ oligomers. Lacor et al. (2004) showed that synthetic Aβ oligomers added to cultures of rat hippocampal neurons colocalized with PSD-95 (a synaptic marker) and induced Arc, a synaptic immediate early gene linked to learning dysfunction. In a subsequent study, Lacor et al. (2007) showed that Aβ oligomers bound specifically to excitatory pyramidal hippocampal neurons, leading to decreased membrane expression of memory-related NMDA and EphB2 receptors, induction of long, thin dendritic spines, decreased spine density, and synaptic deterioration.

Effects of Aβ oligomers on synaptic function

Klyubin et al. (2008) reported that Aβ oligomers isolated from the cultured medium of cells expressing Amyloid Precursor Protein (APP) with a familial AD mutation injected ICV into rats completely inhibited the induction of Long-Term Potentiation (LTP) in the CA1-CA3 regions of the hippocampus. Similar effects were observed with Aβ dimers enriched from the CSF of 4 normal human subjects and 2 patients with AD, whereas Aβ monomers had no effect on LTP. In a review of the data supporting the role of various types of Aβ aggregates in AD-related synaptotoxicity and memory disruption, Klyubin et al. (2012) noted that the inhibition of LTP by Aβ dimers requires assembly into larger protofibril-like aggregates. Wang et al. (2017) showed LTP in mouse hippocampal slices was inhibited after incubation with water-soluble AD brain extracts containing Aβ monomers and oligomers, but not after treatment with normal control or Aβ-immunodepleted AD brain extracts. Further studies showed the inhibition of LTP by AD brain extracts was associated with increased presynaptic glutamate release, increased excitatory synaptic input and reduced GABAergic inhibitory input on CA1 pyramidal neurons.

Effects of anti-Aβ oligomer antibodies on neuronal structure and function

Klyubin et al. (2008) showed that peripherally administered antibodies against Aβ oligomers were able to prevent the inhibition of LTP by ICV administered Aβ oligomers or dimers. Pradier et al. (2018) showed that the anti-Aβ protofibril mAb, SAR228810, and/or its murine precursor, SAR255952, inhibited Aβ oligomer-induced decreases in neurite networks in cultures of mouse hippocampal neurons. Gibbs et al. (2019) showed that muPMN310, an anti-Aβ oligomer mAb, inhibited the neurotoxicity of Aβ oligomers in vitro (in cultures of mouse cortical neurons) and in vivo (ICV administration reduced the increases in TNFα [a proinflammatory marker], reductions in synaptic markers [PSD-95 and SNAP25], and deficits in performance on a novel object recognition test observed in mice one week after ICV injection of Aβ oligomers). Finally, as described more fully in the next section of the review, Kastanenka et al. (2016) showed that administration of the murine variant of aducanumab (^{ch}12F6A) to 18-month old Tg2576 mice for 2 weeks resulted in a substantial reduction in the percentage of cortical neurites with calcium overload.

Conclusion

The nonclinical information submitted supports the argument that direct interaction of aducanumab with Aβ oligomers may reduce adverse effects of the oligomers on neuronal structure and function, potentially leading to acute treatment effects in patients with Alzheimer's disease. However, the extensive heterogeneity of Aβ aggregates (differences in the size, number, conformation, and post-translational modifications of component monomers) has complicated their characterization and the elucidation of their roles in causing the acute and chronic symptoms and the pathology of Alzheimer's disease.

4.2 Secondary Pharmacology

No secondary pharmacology studies of aducanumab were submitted.

4.3 Safety Pharmacology

No stand-alone safety pharmacology studies were submitted. Safety pharmacology was assessed in the pivotal toxicology studies of aducanumab in cynomolgus monkeys and of aducanumab and ^{ch}12F6A in aged Tg2576 mice. There were no adverse effects on ECG in the 4-week monkey study or on CNS- or pulmonary-related clinical signs in either species.

5 Pharmacokinetics/ADME/Toxicokinetics

5.1 PK/ADME

Pharmacokinetics (PK) of Chimeric 12F6A IgG2a (ch12F6A) in Tg2576 and Aged Wild-Type Mice Following a Single Intravenous (IV), Intraperitoneal (IP) or Subcutaneous (SC) Injection

(Biogen Idec Study P037-10-02; Final Report, November 10, 2010; non-GLP)

Aged Tg2576 and wild-type (WT) mice were administered single doses of ^{ch}12F6A at 2 mg/kg or 10 mg/kg IV, IP, or SC, as shown in the table below:

Treatment group	Animal Info	Test Article Lot#	Dose Formulation	Dose Level mg/kg	Dose Volume mL/kg	Route
A	Tg2576	PDR-105-09-265	0.4 mg/ml in formulation buffer*	2	5	IV
B	Tg2576			2	5	IP
C	Tg2576			2	5	SC
D	Tg2576		2 mg/ml in formulation buffer	10	5	IV
E	Tg2576			10	5	IP
F	Tg2576			10	5	SC
G	Wt		0.4 mg/ml in formulation buffer	2	5	IV
H	Wt			2	5	IP
I	Wt			2	5	SC

Blood samples were collected from 4 animals per time point, predose and at 0.083, 2, 6, 24, 48, 96, 168, 216, and 264 hours postdose for determination of ^{ch}12F6A concentration in plasma by ELISA.

As shown in the summary tables below, administration of 2 mg/kg IV ^{ch}12F6A resulted in slightly greater terminal half-life and slightly slower clearance rate in WT mice compared to Tg2576 mice, consistent with the possibility that binding to the target (aggregated amyloid) in the brain may have enhanced antibody clearance from the plasma in Tg2576 mice. Steady state volume of distribution was similar in Tg2576 and WT mice and was approximately equal to blood volume. T_{max} was immediately after dosing for IV, 2-6 hours postdose for IP, and 24 hours postdose for SC administration. Bioavailability was approximately 100% after IP and SC administration. Plasma

exposures (AUC) increased approximately dose-proportionally from 2 mg/kg to 10 mg/kg for all routes of administration in Tg2675 mice.

Table 1. BIIB037 Murine Chimeric Plasma Exposure and Pharmacokinetics - Intravenous Administration

Group	Route	Dose (mg/kg)	Species	C0 (ug/mL)	Cmax +/- SE (ug/mL)	Clast (ug/mL)	AUCall +/- SE (hr*ug/mL)
A	iv	2	Tg2576	66.0	65.0 +/- 5.17	3.01	2420 +/- 170
D	iv	10	Tg2576	343	337 +/- 44.1	5.15	10800 +/- 304
G	iv	2	Wild Type	73.0	72.1 +/- 4.73	5.11	2840 +/- 202

Group	Route	Dose (mg/kg)	Species	Half Life (hr)	AUCINF (hr*ug/mL)	AUCINF %Extrap	CL (mL/hr/kg)	Vss (mL/kg)	Vz (mL/kg)
A	iv	2	Tg2576	94.7	2830	14.5	0.706	90.9	96.4
D	iv	10	Tg2576	89.6	11500	5.8	0.871	102	113
G	iv	2	Wild Type	123	3750	24.2	0.533	91.6	94.5

Table 2. BIIB037 Murine Chimeric Plasma Exposure and Pharmacokinetics - Extravascular Administration

Group	Route	Dose (mg/kg)	Species	Tmax (hr)	Cmax +/- SE (ug/mL)	Clast (ug/mL)	AUCall +/- SE (hr*ug/mL)
B	ip	2	Tg2576	2.0	20.5 +/- 4.52	2.66	2000 +/- 99.5
C	sc	2	Tg2576	24.0	33.9 +/- 17.2	4.81	2410 +/- 623
E	ip	10	Tg2576	6.0	127 +/- 27.2	4.78	12400 +/- 814
F	sc	10	Tg2576	24.0	80.3 +/- 10.9	20.7	14100 +/- 442
H	ip	2	Wild Type	2.0	35.0 +/- 12.1	2.95	3030 +/- 649
I	sc	2	Wild Type	24.0	16.9 +/- 2.11	3.98	3330 +/- 112

Group	Route	Dose (mg/kg)	Species	AUCINF (hr*ug/mL)	AUCINF %Extrap	Half Life (hr)	Bioavailability (extravas AUCINF/ intraven AUCINF)
B	ip	2	Tg2576	2420	17.4	110	0.86
C	sc	2	Tg2576	3100	22.1	99.0	1.10
E	ip	10	Tg2576	13000	5.00	95.0	1.13
F	sc	10	Tg2576	18700	24.4	152	1.63
H	ip	2	Wild Type	3540	14.4	120	0.94
I	sc	2	Wild Type	4340	23.2	175	1.16

(pages 8-9 of Study Report)

5.2 Toxicokinetics

Toxicokinetic data for aducanumab were reviewed with the toxicity studies.

5.3 Methods of Analysis

The analytical methods, qualification, and validation studies (listed in Section 3.1) for the determination of aducanumab, ^{ch}12F6A, and anti-drug antibody (ADA)

concentrations in plasma or serum from mouse, rat, and/or monkey were reviewed and found to adequately support the conclusions of the pivotal toxicology studies conducted in Tg2576 mouse, Sprague Dawley rat, and cynomolgus monkey.

6 General Toxicology

6.1 Single-Dose Toxicity

No single-dose toxicity studies were submitted.

6.2 Repeat-Dose Toxicity

BIIB037: A 4 Dose, Once Weekly Subcutaneous Injection Tolerability and Toxicokinetic Study in Cynomolgus Monkeys

(Biogen Idec Study P037-15-01; Final Report, April 11, 2016; non-GLP; non-QA)

Three male cynomolgus monkeys were administered 250 mg/kg aducanumab via SC injection at 5 mL/kg on Days 1, 8, 15, and 22. Vehicle (b) (4) 150 mM L-arginine HCl, and 0.05% [w/v] polysorbate 80, pH (b) (4) was injected SC on the contralateral side on each day of dosing for comparison of local toxicity. Parameters evaluated included mortality, clinical signs, body weights, Draize scoring of injection sites, food consumption, and toxicokinetics.

There were no aducanumab-related effects. Plasma aducanumab exposures on Day 22 were: C_{max}= 1236 ± 205 µg/mL and AUC_{last} = 22235 ± 2064 µg*hr/mL.

BIIB037: A 4-Week Tolerability and Toxicokinetic Study when Administered by Intravenous and Subcutaneous Injection to Cynomolgus Monkeys

(Biogen Idec Study P037-16-01; Final Report, January 13, 2017; GLP; QA)

Male cynomolgus monkeys (3/group) were administered aducanumab at 300 mg/kg via IV bolus or SC injection at 1.89 mL/kg on Days 1, 8, 15, and 22. Vehicle (16.2 mM L-histidine monohydrate, 3.8 mM L-histidine free base, 150 mM L-arginine HCl, 10 mM methionine, and 0.05% [w/v] polysorbate 80, pH 5.5) was injected SC on the contralateral side of SC-dosed animals on each day of dosing for comparison of local toxicity. Parameters evaluated included mortality, clinical signs, physical exam, body weights, Draize scoring of SC injection sites, food consumption, and toxicokinetics.

Animal #2001 showed Grade 1 (very slight/barely perceptible) edema and erythema at the SC injection site at 24 and 48 hours after the 3rd dose and 24 hours after the 4th dose; no effects were seen at the vehicle injection sites. Histopathological evaluation of punch biopsies of the injection sites of Animal #2001 showed mild focal neutrophilic and mononuclear cellular infiltration and hemorrhage at the injection site used for the 4th aducanumab dose; there were no abnormal findings at the injection site used for the 3rd

aducanumab dose. The findings in Animal #2001 were non-adverse and of uncertain relationship to aducanumab.

There were no aducanumab-related adverse effects in any animals. Peak aducanumab concentrations occurred between 0.083 and 2 hours after IV dosing and between 12 and 24 hours after SC dosing. Plasma aducanumab Day 22 C_{max}= 7070 µg/mL (IV) and 2490 µg/mL (SC); AUC_{last} = 324000 µg*hr/mL (IV) and 243000 µg*hr/mL (SC). Absolute bioavailability for SC administration was 75.1% on Day 22.

BIIB037: 4 Week Toxicity Study of BIIB037 When Administered by Slow Intravenous Bolus Injection to Cynomolgus Monkeys with an 8-Week Recovery Period

(Biogen Idec Study P037-09-04; Final Report, December 9, 2010; Amendment No. 1, June 12, 2015; Aducanumab Lot PDR-105-10-54; GLP; QA; signed pathology report; see detailed review by Dr. Wilcox, February 3, 2012, under IND 106230)

Cynomolgus monkeys (4/sex/group main-study, 2/sex Con, MD, and HD 8-week recovery, 79-80 weeks old) were administered vehicle control (b) (4) 150 mM L-arginine HCl, and 0.05% [w/v] polysorbate 80) or aducanumab (Lot PDR-105-10-54; 10, 100, or 300 mg/kg) via slow bolus IV injection at 6 mL/kg weekly for 4 weeks (Days 1, 8, 15, 22, and 29). Aducanumab 300 mg/kg was a maximum feasible dose based on use of the 50 mg/mL clinical formulation and a maximum dose volume of 6 mL/kg for a slow IV bolus in the monkey. Parameters evaluated included dosing formulation analyses, mortality, clinical observations, body weight, food consumption, ophthalmic examinations, ECG, hematology, coagulation, clinical chemistry, urinalysis, toxicokinetics, immunogenicity, necropsy, organ weights, and histopathology.

There were no aducanumab-related effects. Microscopic findings at injections sites were similar in all groups, including controls, and were not present after the 8-week recovery period. The NOAEL (300 mg/kg/day) was associated with plasma aducanumab exposures on Day 22 of $AUC_{0\text{-}168\ hrs}$ = 338000 µg*hr/mL M, and 358000 µg*hr/mL F. There were no ADA-positive samples.

BIIB037: A 13-Week Toxicity Study Administered by Weekly Slow Bolus Injection to Tg2576 Mice With a 6-Week Recovery Period

(Biogen Idec Study P037-09-02; Final Report, December 9, 2010; GLP; QA; signed pathology report; see detailed review by Dr. Wilcox, February 3, 2012, under IND 106230)

Tg2576 mice (11-14/sex/group main-study, 5/sex/group 6-week recovery, 79-80 weeks old) were administered vehicle control (b) (4) 150 mM L-arginine HCl, and 0.05% [w/v] polysorbate 80), ^{ch}12F6A (10, 70, or 500 mg/kg), or aducanumab (500 mg/kg) via slow bolus IV injection weekly for 13 weeks. Aducanumab 500 mg/kg was a maximum feasible dose based on use of the 50 mg/mL clinical

formulation and a maximum dose volume of 10 mL/kg for a slow IV bolus in the mouse. Parameters evaluated included dosing formulation analyses, mortality, clinical observations, body weight, food consumption, ophthalmic examinations, hematology, clinical chemistry, toxicokinetics, immunogenicity, necropsy, organ weights, histopathology, and microhemorrhage image analysis of the brain. The high dose was a maximum feasible dose based on use of the 50 mg/mL clinical formulation and a maximum dose volume of 10 mL/kg for a slow IV bolus in the mouse.
Dose-related effects in ^{ch}12F6A-dosed groups included increased incidence and/or severity of meningeal vascular inflammation (minimal in 1/10 Con F, 1/10 LDM; mild in 1/9 MDM, 1/10 MDF, 2/10 HDM; moderate in 1/10 HDF), thrombosis (minimal to mild in 2/10 HDM), acute hemorrhage (mild in 1/10 MDM), and perivascular lymphocytic infiltration (minimal in 1/10 LDM, 2/9 MDM, and 1/10 MDF; minimal to mild in 2/10 HDF) in the brain. Recovery groups showed meningeal vascular inflammation (minimal in 1/5 MDF, 1/5 HDF). The analysis of brain microhemorrhages showed slight increases in HDM ^{ch}12F6A and HDF aducanumab groups at termination and after the 6-week recovery period, compared to controls. Based on the increases in meningeal vascular inflammation and acute hemorrhage in brain at the MD of 70 mg/kg/week (Day 92 AUC_{TAU} = 103000 µg*hr/mL in M, and 74000 µg*hr/mL in F), the NOAEL was the LD of 10 mg/kg/week ^{ch}12F6A (Day 92 AUC_{TAU} = 11000 µg*hr/mL in M, and 10300 µg*hr/mL in F). Anti-drug antibody (ADA) effects were limited to reduced aducanumab plasma levels in 2 animals given 500 mg/kg aducanumab (0 and 2.43 µg/mL, predose on Day 36), compared to those in the ADA-negative mice in the same dose group (34.8, 83.4, 93.0, and 165 µg/mL).

BIIB037: A 6 Month Study of BIIB037 by Weekly Intravenous Injection in the Tg2576 Mouse with a 6 Week Recovery Period

Study no.:	Biogen Idec Study P037-11-01
Study report location:	edr
Date of study initiation:	September 28, 2011
GLP compliance:	Yes, except for stability testing, peer review of histopathology, microhemorrhage analysis, and potency analysis
QA statement:	Yes
Signed pathology report:	Yes
Drug, lot #, and % purity:	BIIB037 Murine Chimeric (^{ch}12F6A IgG2a) Lot PDR-105-11-212, 90.1% pure BIIB037 (human 12F6A IgG1) Lot 3-FIN-0901, 90.5% pure Lot 3-FIN-1007, 91.5% pure

Key Study Findings

- There were no clear drug-related effects in aged Tg2576 mice following IV administration of ^{ch}12F6A (10, 40, or 250 mg/kg) or aducanumab (250 mg/kg) weekly for 6 months.

Methods

Doses:	0, 10, 40, and 250 mg/kg BIIB037 Murine Chimeric (^{ch}12F6A); 250 mg/kg/week BIIB037 (aducanumab)
Frequency of dosing:	Weekly for 6 months
Route of administration:	Slow bolus IV injection via the tail vein
Dose volume:	5 mL/kg
Formulation/Vehicle:	(b) (4), 150 mM L-Arginine HCl, 0.05% (w/v) Polysorbate 80, pH (b) (4)
Species/Strain:	Tg2576 transgenic mouse, (b) (4)
Number/Sex/Group:	10/sex/group main-study; 5/sex/group 6-week recovery period
Age:	16-17 months old at initiation of dosing
Weight:	23.4-46.8 g M; 14.9-33.9 g F
Satellite groups:	Toxicokinetics; 9/sex Con, 31-36/sex/group mAb
Unique study design:	Aged Tg2576 mice were used because they have brain amyloid plaques and cerebral amyloid angiopathy. Analyses of anti-therapeutic antibodies and brain microhemorrhages were included.
Deviation from study protocol:	No deviations were reported that affected study validity or interpretation

Observations and Results

Dosing Solution Analysis

Dosing solutions were all within acceptance criteria.

Mortality

Mortality was assessed twice daily.

No ^{ch}12F6A- or aducanumab-related effects were observed.

Clinical Signs

Cage-side observations were recorded daily. Detailed observations were recorded weekly. Physical exams were conducted weekly.

No ^{ch}12F6A- or aducanumab-related effects were observed.

Body Weights

Body weight was assessed weekly.

No ^{ch}12F6A- or aducanumab-related effects were observed.

Food Consumption

Food consumption was assessed weekly.

No ^{ch}12F6A- or aducanumab-related effects were observed.

Ophthalmoscopy

Ophthalmoscopic examinations were conducted twice predose and in Week 25/26 of the dosing period.

No ^{ch}12F6A- or aducanumab-related effects were observed.

Hematology and Clinical Chemistry

Blood was collected from main-study and recovery animals at necropsy or unscheduled euthanasia for hematology and clinical chemistry.

No ^{ch}12F6A- or aducanumab-related effects were observed.

Gross Pathology

All animals surviving to scheduled termination were necropsied at the terminal sacrifice (Day 184 of the dosing phase) or at the end of the recovery period (Day 225). Necropsies were conducted using standard procedures.

No ^{ch}12F6A- or aducanumab-related effects were observed.

Organ Weights
Organs were isolated and weighed using standard procedures.

No ^{ch}12F6A- or aducanumab-related effects were observed.

Microhemorrhage Analysis
Following necropsy, main-study Control, HD ^{ch}12F6A, and aducanumab groups were evaluated for the number and area of brain microhemorrhages using Perls staining for hemosiderin, the degradation product of hemoglobin.

The number of brain microhemorrhages present in the sections analyzed was not statistically significantly different between Control, HD ^{ch}12F6A, and aducanumab groups.

Histopathology
Histopathology was conducted on all Control and HD main-study and recovery animals using standard procedures; evaluation of MD and LD animals was limited to gross lesions and target tissues (brain). The battery of tissues examined was adequate. Peer review was conducted. A signed pathology report was provided.

Histological Findings
As shown in the summary tables below, there were slight increases in the incidence and/or severity of meningeal and cerebral vascular inflammation and thickening in the brain in females at 40 mg/kg/week ^{ch}12F6A, with no further increases at 250 mg/kg/week. No consistent dose-related effects were seen following the 6-week recovery period. The Pathology Report stated that “These changes were considered suggestive of a possible exacerbation of the disease model-related vascular inflammation…” *(page 1801-1802 of Study Report)*. However, based on the presence of minimal findings in controls and the lack of consistent dose-dependence, the slight increases in the incidence and/or severity of brain vascular inflammation and thickening in MD and HD females were not clearly drug related.

Text Table 6 Selected Histopathologic Findings in the Brain of Main Study Animals (Day 184)

	Males					Females				
Group	1	2	3	4	5	1	2	3	4	5
Dose (mg/kg/dose)*	0	10	40	250	250	0	10	40	250	250
No. animals examined	10	9	10	10	10	10	10	10	10	10
Inflammation/infiltrate, mixed/lymphocytic, vascular	**3**	**4**	**1**	**4**	**1**	**1**	**2**	**4**	**3**	**1**
Minimal	3	4	1	4	1	1	2	2	2	1
Mild	0	0	0	0	0	0	0	2	1	0
Thickening, vascular	**0**	**1**	**2**	**2**	**1**	**1**	**1**	**5**	**3**	**0**
Minimal	0	1	2	2	1	1	1	3	1	0
Mild	0	0	0	0	0	0	0	2	2	0
Pigmentation, hemosiderin	**1**	**0**	**0**	**1**	**1**	**0**	**0**	**0**	**1**	**0**
Minimal	1	0	0	1	1	0	0	0	1	0
Thrombosis, acute	**0**	**1**	**0**	**0**	**0**	**0**	**0**	**0**	**1**	**0**
Minimal	0	1	0	0	0	0	0	0	1	0

* = Groups 2-4 BIIB037 Murine Chimeric (12F6A Ig2a), Group 5 BIIB037.
Bold Number=Number of animals with the finding.

(page 1800 of Study Report)

Text Table 7 Selected Histopathologic Findings in the Brain of Recovery Animals (Day 225)

	Males					Females				
Group	1	2	3	4	5	1	2	3	4	5
Dose (mg/kg/dose)*	0	10	40	250	250	0	10	40	250	250
No. animals examined	5	5	5	5	5	5	5	5	5	5
Necrosis, white matter	**0**	**2**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**
Mild	0	2	0	0	0	0	0	0	0	0
Infiltrate, macrophage (gitter cell), white matter	**0**	**2**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**
Mild	0	2	0	0	0	0	0	0	0	0
Inflammation/infiltrate, mixed/lymphocytic, vascular	**2**	**2**	**3**	**3**	**0**	**1**	**4**	**1**	**1**	**1**
Minimal	2	1	2	3	0	0	4	1	0	1
Mild	0	0	1	0	0	1	0	0	1	0
Moderate	0	1	0	0	0	0	0	0	0	0
Thickening, vascular	**3**	**4**	**4**	**3**	**2**	**0**	**2**	**1**	**0**	**1**
Minimal	3	2	2	3	2	0	1	0	0	1
Mild	0	1	2	0	0	0	1	1	0	0
Moderate	0	1	0	0	0	0	0	0	0	0
Pigmentation, hemosiderin	**0**	**2**	**2**	**1**	**0**	**0**	**0**	**0**	**1**	**1**
Minimal	0	2	2	1	0	0	0	0	1	1

* = Groups 2-4 BIIB037 Murine Chimeric (12F6A Ig2a), Group 5 BIIB037.
Bold Number=Number of animals with the finding.

(page 1801 of Study Report)

Toxicokinetics

Blood samples were collected from TK animals (3/sex/group/time point) on Days 1 and 183, at 0 and 8 hours postdose for controls, and at 0, 8, 24, 72, and 168 hours postdose (+ 366 hours postdose Day 183) for ^{ch}12F6A and aducanumab groups; and at necropsy for main-study and recovery animals.

No ^{ch}12F6A or aducanumab was detected in control or predose plasma samples, except for one Con F Recovery animal that showed 1.5 µg/mL ^{ch}12F6A on Day 225 for unknown reasons. ^{ch}12F6A exposures (C_{max} and AUC_{TAU}) increased roughly dose-proportionally on Day 1, except for Cmax in F, which increased greater than dose-proportionally. On Day 183, C_{max} increased dose-proportionally in M, and less than dose-proportionally in F, while AUC_{TAU} increased less than dose-proportionally in M and

F. Exposures were generally similar in males and females. Moderate accumulation was observed at 10 and 40 mg/kg/week ^{ch}12F6A over the 6-month dosing period. There was no accumulation at the 250 mg/kg/week dose of ^{ch}12F6A or aducanumab.

Table 2. Group Mean BIIB037 and BIIB037 Murine Chimeric Toxicokinetic Summary Data; Sorted by Compound, Day, Group, Dose, and Gender

Compound	Day	Group	Dose (mg/kg)	Gender	C_0 (µg/mL)	C_{max} (µg/mL)	SE C_{max} (µg/mL)	C_{avg} (µg/mL)	C_{min} (µg/mL)	AUC_{TAU} (µg*h/mL)	CL_{SS} (mL/h/kg)	V_{SS} (mL/kg)	$HL_{\lambda z}$ (h)
BIIB037	1	5	250	Female	3370	2190	225	597	108	100000	NR	NR	NR
				Male	5270	3000	155	689	117	116000	NR	NR	NR
				F + M	4290	2600	218	642	113	108000	NR	NR	NR
	183	5	250	Female	3840	2290	276	625	152	105000	2.38	157	61.8
				Male	5790	3220	209	734	143	123000	2.03	131	104
				F + M	4800	2750	259	679	147	114000	2.19	141	81.2
BIIB037 Murine Chimeric	1	2	10	Female	92.6	75.5	8.61	34.4	15.3	5780	NR	NR	NR
				Male	166	122	16.0	44.2	16.7	7430	NR	NR	NR
				F + M	129	98.9	13.3	39.3	15.9	6600	NR	NR	NR
		3	40	Female	634	486	51.5	180	82.5	30200	NR	NR	NR
				Male	740	490	48.9	175	81.8	29400	NR	NR	NR
				F + M	682	488	31.8	177	82.1	29700	NR	NR	NR
		4	250	Female	3360	2630	269	999	341	168000	NR	NR	NR
				Male	2600	2300	317	1090	446	183000	NR	NR	NR
				F + M	2960	2470	200	1040	393	175000	NR	NR	NR
	183	2	10	Female	118	100	19.0	60.6	34.2	10200	0.983	122	63.1
				Male	85.8	98.0	25.4	63.8	42.4	10700	0.933	149	101
				F + M	97.3	92.9	17.5	62.1	38.3	10400	0.959	135	81.9
		3	40	Female	511	449	40.1	222	68.5	37200	1.07	104	79.3
				Male	570	451	162	227	135	38200	1.05	171	117
				F + M	538	450	74.6	224	102	37700	1.06	137	98.0
		4	250	Female	1540	1470	601	678	370	114000	2.19	233	57.4
				Male	2540	2120	501	1020	473	172000	1.45	188	100
				F + M	2030	1800	380	851	422	143000	1.75	208	86.4

(page 1229 of Study Report; TAU = dosing interval, 0-168 hours)

Anti-Drug Antibody (ADA) Analysis

Blood was collected from TK animals for ADA analysis prior to dosing on Day 1, and 336 hours postdose on Day 183; and at necropsy from main-study and recovery animals.

ADA analysis was positive in 3/31 samples from aducanumab-dosed animals (one predose, one 336 hr postdose Day 185, and one at recovery sacrifice on Day 255) and in 6/65 ^{ch}12F6A-dosed animals (two from the vehicle group, one from a MD animal predose on Day 1, and the remaining three from MD and HD animals 336 hours postdose on Day 183). Both ADA-positive aducanumab-dosed animals showed reduced (~65%) plasma aducanumab exposures compared to other animals in this dose group. Similarly, the two HD ADA-positive ^{ch}12F6A-dosed animals showed reductions of ~65% in plasma ^{h}12F6A exposure. Based on the small number of animals affected, the impact of ATA on the results of the study was negligible.

7 Genetic Toxicology

No genotoxicity studies of aducanumab were conducted because antibodies are generally unable to interact with genetic material.

8 Carcinogenicity

Carcinogenicity studies of aducanumab were not conducted because its target, aggregated Aβ, is not present in wild-type rodents. No increases in the incidence or severity of proliferative lesions were observed in the 4-week study in monkey or the 13- or 26-week studies in aged Tg2576 mouse. The Division informed the sponsor that carcinogenicity studies of aducanumab would not be required to support a Biologic Licensing Application (see email of June 14, 2016, and review of sponsor's rationale for not conducting carcinogenicity studies by Dr. Wilcox, IND 106230, June 23, 2020). The effect of aducanumab administration on the risk of malignancy in humans is unknown.

9 Reproductive and Developmental Toxicology

9.1 Fertility and Early Embryonic Development

BIIB037: A Once Weekly Intravenous Injection Fertility and Early Embryonic Development Study with a Toxicokinetic Phase in Male and Female Sprague Dawley Rats

Study no.:	Biogen Idec Study P037-16-04
Study report location:	docuBridge
Date of study initiation:	September 9, 2016
GLP compliance:	Yes, except for pre-study analyses of aducanumab
QA statement:	Yes
Drug, lot #, and % purity:	Aducanumab Lot TR-PPD-018851, purity 94.1%

Key Study Findings

- The NOAEL for male and female systemic toxicity, reproductive performance, fertility, and early embryonic toxicity was the HD of 1000 mg/kg/week IV aducanumab (Day 49 AUC_{last} = 509000 µg*hr/mL M, 471000 µg*hr/mL F).

Methods

Doses:	0, 100, 300, 1000 mg/kg/week aducanumab
Frequency of dosing:	M: Weekly for 5 doses prior to mating, throughout mating, continuing until euthanasia on Day 61 or 62 (9 doses total) F: Weekly for 3 doses prior to mating, throughout mating, continuing until GD 7 (4 to 6 doses total); euthanized GD15
Dose volume:	8 mL/kg
Route of administration:	IV injection, slow push over 1-2 minutes
Formulation/Vehicle:	16.2 mM L-Histidine monohydrate, 3.8 mM L-histidine free base, 150 mM L-Arginine HCl, 10 mM Methionine, 0.05% (w/v) polysorbate 80, pH 5.5
Species/Strain:	Crl:CD(SD) Sprague Dawley rat
Number/Sex/Group:	25 M and 25 F per group
Satellite groups:	TK satellite groups at 0 (3/sex), 3, 15, and 25 mg/kg (6/sex/group)
Study design:	Based on TK results, ADA analyses were not conducted
Deviation from study protocol:	No deviations were reported that affected study validity or interpretation

Observations and Results

Dosing Solution Analysis

Samples of the first and last dosing formulations administered were analyzed for aducanumab concentration using validated methods.

All samples of dosing solutions were within 10% of the nominal concentrations. No aducanumab was detected in the placebo control dosing solutions.

Toxicokinetics

Blood samples were collected following the first and final doses from 3 TK Control animals per sex at 0 (pre-dose) and 5 minutes postdose, and from 3/sex/time point TK aducanumab-dosed animals at 0, 5 minutes, and 2, 8, 12, 24, 96, and 168 hours postdose. Serum samples were analyzed for aducanumab concentration using a validated method.

As shown in the summary table below, serum aducanumab exposures increased slightly less than dose proportionally and increased 1.4- to 1.73-fold with repeated weekly dosing from Day 0 to Day 49.

Text Table 8
Toxicokinetic Summary

Dose		100 mg/kg/dose			300 mg/kg/dose			1000 mg/kg/dose		
Number of animals		M (6)	F (6)	Combined	M (6)	F (6)	Combined	M (6)	F (6)	Combined
Day	Parameter									
0	Cmax (µg/mL)	2280	1850	2100	5770	4350	5060	13,000	18,900	16,000
	AUClast (µg*hr/mL)	53,800	45,500	49,700	127,000	115,000	121,000	336,000	338,000	337,00
49	Cmax (µg/mL)	3280	2740	3010	6770	5080	5925	24,000	22,300	23,150
	AUClast (µg*hr/mL)	80,100	70,900	75,500	220,000	184,000	202,000	509,000	471,000	490,000

(page 24 of Study Report)

Mortality

All animals were observed for mortality twice daily throughout the study.

No drug-related effects on mortality were observed.

Clinical Signs

Cageside observations were conducted at least once daily during the dosing period.

Single incidents of rales were observed in 1/25 MDM, 6/25 HDM, and 2/25 HDF approximately 30 minutes postdose between the 4th and 9th doses; one HDM had rales following the 6th and 7th doses. These effects were drug-related but not adverse because they did not persist.

Body Weight

Body weight was recorded twice weekly during the dosing period.

No aducanumab-related effects were observed.

Food Consumption

Food consumption was recorded on a per cage basis twice weekly until cohabitation, and then on GD 0, 3, 7, 10, 13, and 15.

No aducanumab-related effects were observed.

Reproductive Performance

Male and female reproductive performance (mating index, fertility index, copulation index, conception index, estrous cycle length, and pre-coital interval) was assessed during the mating period.

No aducanumab-related effects were observed.

Hematology and Coagulation

Blood samples were collected from main-study animals at termination. Standard parameters were evaluated.

No aducanumab-related effects were observed.

Clinical Chemistry

Blood samples were collected from main-study animals at termination. Standard parameters were evaluated.

No aducanumab-related effects were observed.

Necropsy, Organ Weights, and Sperm and Fertility Analyses

All main-study animals were euthanized and necropsied at scheduled termination (Day 61 or 62 for M, GD 15 for F). Tissues collected and fixed included coagulating glands, ovaries and oviducts, pituitary glands, prostate glands, injection sites, seminal vesicles, testis with epididymis and vas deferens, uterus with cervix and vagina, and all gross lesions. Organs weighed included brain, testes, epididymides, ovaries, and pituitary gland. Sperm analysis conducted on all main-study males included sperm motility, viability, and morphology, epididymal and testicular sperm concentrations per gram of tissue, and sperm production rate. Fertility parameters evaluated included number of corpora lutea, number and viability of embryos, early resorptions, and uterine implantation sites. Histopathology was not conducted because there were no adverse effects on reproductive parameters.

Dark red discolored mandibular lymph nodes were observed in 2/25 MDM and 4/25 HDM. One HDM also showed enlarged mandibular lymph nodes. These effects were not adverse because they did not correlate with any other toxicities.

9.2 Embryofetal Development

BIIB037: An Intravenous Injection Embryo/Fetal Development Study with a Toxicokinetic Phase in Female Sprague Dawley Rats

Study no.:	Biogen Idec Study P037-17-01
Study report location:	docuBridge
Date of study initiation:	January 4, 2018
GLP compliance:	Yes, except for pre-study analyses of aducanumab and control
QA statement:	Yes
Drug, lot #, and % purity:	Aducanumab Lot VR0014, purity 97.0%

Key Study Findings

- The NOAEL for maternal and developmental toxicity was the HD of 1000 mg/kg/week IV aducanumab (GD 13 AUC_{0-t} = 274000 µg*hr/mL)

Methods

Doses:	0, 100, 300, 1000 mg/kg/week aducanumab
Frequency of dosing:	GD 6 and GD 13
Dose volume:	10 mL/kg
Route of administration:	IV injection, slow push over 1-2 minutes
Formulation/Vehicle:	16.2 mM L-Histidine monohydrate, 3.8 mM L-histidine free base, 150 mM L-Arginine HCl, 10 mM Methionine, 0.05% (w/v) polysorbate 80, pH 5.5
Species/Strain:	Crl:CD(SD) Sprague Dawley Rat
Number/Sex/Group:	25 F/group
Satellite groups:	TK (3 F Con, 9 F/group aducanumab-dosed)
Study design:	ADA samples were not analyzed
Deviation from study protocol:	No deviations were reported that affected study validity or interpretation

Observations and Results

Dosing Solution Analysis

Samples of formulations used for dosing on GD 5 were analyzed for aducanumab using validated methods.

All dosing solutions were within 1% of the nominal concentrations.

Toxicokinetics

Blood samples were collected from TK animals on GD 5 and GD 13 at 0 and 5 minutes postdose for controls, and at 0, 5 minutes, and 2, 8, 12, 24, 96, and 168 hours postdose for aducanumab-dosed groups. Maternal and fetal blood samples were collected at termination of the TK animals on GD 20. Serum samples were analyzed for aducanumab concentrations using validated methods.

Aducanumab was not detected in samples from controls or in predose samples from aducanumab-dosed animals. As shown in the summary tables below, serum aducanumab exposures increased less than dose-proportionally and were similar on GD 13 and GD 6. On GD 20 (7 days after the final dose) aducanumab was measurable in maternal and fetal serum samples.

Text Table 10
Toxicokinetic Parameters for BIIB037 in Maternal Rats

BIIB037 Dose (mg/kg/dose):	**100**	**300**	**1000**
Parameter (Units)		Gestation Day 6	
AUC(0-t) (µg ·h/mL)	47,600	109,000	293,000
AUC(0-t)/D	476	364	293
Cmax (µg /mL)	1580	5040	17,700
Cmax/D	15.8	16.8	17.7
Tmax (h)	0.083	0.083	0.083
T1/2 (h)	64.9	79.6	60.2
		Gestation Day 13	
AUC(0-t) (µg ·h/mL)	44,500	101,000	274,000
AUC(0-t)/D	445	338	274
Cmax (µg /mL)	1980	5300	18,700
Cmax/D	19.8	17.7	18.7
Tmax (h)	0.083	0.083	0.083
T1/2 (h)	47.3	47.9	42.1
Accumulation AUC(0-t) Ratio	0.935	0.929	0.933

Text Table 9
Mean Fetal and Maternal BIIB037 Serum Concentrations on GD 20

Group	Treatment (mg/kg/dose)	GD	Subject	Concentration (µg/mL)		Maternal:Fetal Concentration Ratio
				Fetal	Maternal	
2	100	20	Mean	73.8	45.6	1.02
			SD	100	7.08	0.36
3	300	20	Mean	81.5	96.8	1.20
			SD	13.4	22.9	0.30
4	1000	20	Mean	124	196	1.60
			SD	24.2	38.4	0.24

(page 21 of Study Report)

Mortality

All animals were observed for mortality twice daily.

No aducanumab-related effects were observed.

Clinical Signs

Clinical observations were recorded daily.

No aducanumab-related effects were observed.

Body Weight

Body weights were recorded on GD 0 and daily from GD 5 to GD 21.

No aducanumab-related effects were observed.

Food Consumption

Food consumption was recorded on GD 0 and daily from GD 5 to GD 21.

No aducanumab-related effects were observed.

Necropsy

Main-study animals were euthanized and necropsied on GD 21. Gravid uterine weight was recorded.

No aducanumab-related effects were observed.

Cesarean Section Data

Ovaries and uterus were examined for number of corpora lutea, implantations sites, fetal number/weight/survival/sex, and early and late resorptions.

No aducanumab-related effects were observed.

Offspring

There were no aducanumab-related effects on the incidence of fetal external, visceral, or skeletal malformations or variations, compared to controls.

9.3 Prenatal and Postnatal Development

BIIB037: An Intravenous Injection Pre- and Postnatal Development Study, Including Maternal Function, in Sprague Dawley Rats

Study number:	Biogen Idec Study P037-17-02
Study report location:	docuBridge
Date of study initiation:	January 4, 2018
GLP compliance:	Yes, except for pre-study analyses of aducanumab
QA statement:	Yes
Drug, lot #, and % purity:	Aducanumab Lot VR0014, purity 97.0%

Key Study Findings

- The HD of 1000 mg/kg/week IV aducanumab was the NOAEL for F_0 maternal and F_1 parental systemic and reproductive toxicity, and for F_1 and F_2 neonatal and developmental toxicity.

Text Table 3
Experimental Design

Group Number	Test Article	Dosage Level (mg/kg/dose)[a]	Dose Concentration (mg/mL)	Dose Volume (mL/kg)	Number of Females
1	Vehicle	0	0	10	25
2	BIIB037	100	10	10	25
3	BIIB037	300	30	10	25
4	BIIB037	1000	100	10	25

[a] No correction factor was used.

(page 16 of Study Report)

Text Table 6
Offspring Allocation for Behavioral Testing and Breeding

Maximum No. Selected (Subset)	Age	Evaluation
25/sex/group (A) [a]	PND 20 and 60	Auditory Startle
25/sex/group (A) [a]	PND 21 and 61	Motor Activity
25/sex/group (B)	PND 22	Learning and Memory [b]
25/sex/group (A) [a]	PND 62	Learning and Memory [b]
25/sex/group (A) [a]	Minimum of 85 days	Breeding

* For each endpoint, a maximum of 25 animals/sex/group were selected and represented 1 pup/sex/litter.
[a] The same pup subset was used for auditory startle, locomotor activity, PND 62 learning and memory and breeding.
[b] Different pups were evaluated on PND 22 and 62.

(page 18 of Study Report; PND = Postnatal Day)

Methods

Doses:	0, 100, 300, 1000 mg/kg
Frequency of dosing:	Weekly from GD 6 to Lactation Day (LD) 21
Route of administration:	IV injection, slow push over 1-2 minutes
Dose volume:	10 mL/kg
Formulation/Vehicle:	16.2 mM L-Histidine monohydrate, 3.8 mM L-histidine free base, 150 mM L-Arginine HCl, 10 mM Methionine, 0.05% (w/v) polysorbate 80, pH 5.5
Species/Strain:	Crl:CD(SD) Sprague Dawley rat
Study design:	See Tables 3 and 6 above
Deviation from study protocol:	No deviations were reported that affected study validity or interpretation

Observations and Results

Dosing Solution Analysis
Samples of the first and last dosing formulations administered were analyzed for aducanumab concentration using validated methods.

All samples of dosing solutions were within 2% of the nominal concentrations.

Pregnant Females (F_0)
Mortality
Mortality/morbidity assessments were conducted twice daily (AM and PM).

No aducanumab-related effects were observed.

Clinical Observations
Detailed clinical observations were recorded once daily.

No aducanumab-related effects were observed.

Body Weight
Body weights were recorded on GD 0, 5, 6, 9, 13, 16, and 20, and daily from LD 0 to 21.

No aducanumab-related effects were observed.

Food Consumption
Food consumption was recorded on GD 5, 6, 9,13, 16, and 20, and LD 0, 1, 4, 7, 11, 14, 18, and 21.

No aducanumab-related effects were observed.

Parturition
On the day parturition was initiated, the number of stillborn and live pups and the gestation length were recorded.

No aducanumab-related effects were observed.

Necropsy
F_0 females reaching parturition were euthanized and necropsied on LD 21. Evaluations included examination of the contents of the thoracic, abdominal, and pelvic cavities, number of implantation sites, gross lesions, and injections sites. F_0 females failing to reach parturition were euthanized on post-mating Day 25 (or within 24 hours of total litter loss) and examined for early implantation loss and corpora lutea, in addition to the parameters listed above.

No aducanumab-related effects were observed.

F_1 Generation
Where possible, litters were standardized on PND 4 to 8 pups/litter of equal sex distribution; remaining offspring were euthanized and discarded. Prior to weaning, 1 pup/sex/litter was assigned to Subset A and 1 pup/sex/litter was assigned to Subset B.

Mortality
Mortality/morbidity assessments were conducted twice daily (AM and PM).

No aducanumab-related effects were observed.

Clinical Observations
Detailed clinical observations were conducted on PND 1, 4, 7, 10, 14, 17, and 21, and weekly following weaning.

No aducanumab-related effects were observed.

Sex Determination
Pups were sexed on PND 0, 4, 14, and 21.

No aducanumab-related effects were observed.

Body Weight
Pups were weighed on PND 1, 4, 7, 10, 14, 17, and 21, and weekly following weaning. F_1 females used for assessment of reproductive toxicity (mating was initiated at PND 85) were weighed on GD 0, 6, 9, 12, 15, 18, and 20, and on LD 1 and 4.

No aducanumab-related effects were observed.

Developmental Landmarks
Pups in Subsets A and B were assessed daily from PND 35 for balanopreputial separation (M) or from PND 25 for vaginal patency (F). Age and body weight were recorded at the age these developmental landmarks were attained.

No aducanumab-related effects were observed.

Auditory Startle Response
Pups in Subset A were assessed for auditory startle response on PND 20 and 60.

No aducanumab-related effects were observed.

Motor Activity
Pups in Subset A were assessed for motor activity on PND 21 and 61.

No aducanumab-related effects were observed.

Learning and Memory
Pups in Subsets B and A were assessed for learning and memory on PND 22 and PND 62, respectively, using a water-filled 8-unit T-maze (Biel maze).

No aducanumab-related effects were observed.

Parturition
On the day parturition was initiated in F_1 females, the number of stillborn and live pups and the gestation length were recorded.

No aducanumab-related effects were observed.

Necropsy
Pups in Subset B were euthanized and necropsied following attainment of sexual developmental landmarks. Pups in Subset A were euthanized and necropsied following mating and parturition (LD 4 for F_1 M and F_1 F reaching parturition). Evaluations included examination of the contents of the thoracic, abdominal, and pelvic cavities, number of implantation sites, gross lesions, and injections sites. F_1 females failing to reach parturition were euthanized on post-mating Day 25 (or within 24 hours of total litter loss) and examined for early implantation loss and corpora lutea, in addition to the parameters listed above.

No aducanumab-related effects were observed.

F_2 Generation

Mortality

Mortality/morbidity assessments were conducted twice daily (AM and PM).

No aducanumab-related effects were observed.

Clinical Observations

Detailed clinical observations were conducted on PND 1 and 4.

No aducanumab-related effects were observed.

Sex Determination

Pups were sexed on PND 0 and 4.

No aducanumab-related effects were observed.

Body Weight

Pups were weighed on PND 1 and 4.

No aducanumab-related effects were observed.

Termination

F_2 pups found dead or euthanized early were euthanized and examined for visceral abnormalities. F_2 pups surviving to termination on PND 4 were euthanized and discarded without further examination.

No aducanumab-related effects were observed.

10 Special Toxicology Studies

Tissue Cross-Reactivity of BIIB037 with Human, Mouse and Cynomolgus Monkey Tissues *Ex Vivo*

(Biogen Idec Study P037-09-01; initiated December 21, 2009; reviewed by Dr. Wilcox, February 3, 2012; GLP; QA)

The potential tissue cross-reactivity of fluorescein-conjugated aducanumab was evaluated in cryosections of human, Tg2576 mouse, and cynomolgus monkey tissues at 0.1 and 0.5 µg/mL using standard immunohistochemical methods.

Moderate to marked staining was observed in Tg2576 mouse brain (positive control), whereas no staining was observed in wild-type B6-SJL mouse brain (negative control). There was no specific staining with an isotype control human IgG antibody. Specific staining of vascular endothelium was observed with an anti-CD31 platelet-endothelial cell adhesion molecule 1 antibody, demonstrating validity of the tissue sections and methods.

In Tg2576 mice, moderate to marked specific staining of fluorescein-conjugated aducanumab was observed in amyloid plaques in the cerebral cortex and hippocampus and to blood vessel walls in these areas and in cerebellum.

In cynomolgus monkey, there was specific cytoplasmic staining in the lamina propria of stomach, medulla of kidney, pars distalis and pars nervosa of pituitary, placenta, prostate, myometrium of uterus, and (equivocally) in presumptive ganglion cells of retina and red pulp of spleen.

In human, specific staining was observed in blood vessel walls in the cerebral cortex (minimal to mild, frequent) and in the fallopian tube (minimal to moderate, rare). There was specific cytoplasmic staining in sclera and ciliary body of eye, fallopian tube, kidney, presumptive hepatic stellate and Kupffer cells in liver, pancreas, testis, and (equivocally) in spleen.

***In Vitro* Evaluation of the Influence of BIIB037 on Human Whole Blood Hemolysis and Plasma Flocculation**
(Biogen Idec Study P037-10-01; initiated May 14, 2010; GLP; QA)

Aducanumab (0.05, 0.5, and 5 mg/mL) was evaluated for hemolytic potential and plasma flocculation by incubation with whole blood from 3 male and 3 female human donors for 1 hour at 37 °C. Hemolysis was evaluated by hematocrit, whole blood and plasma hemoglobin concentrations, plasma hemolytic index, and visual macroscopic hemolysis assessment. Flocculation was determined by plasma turbidity index and visual flocculation assessment.

No significant hemolysis or flocculation was observed in any of the samples incubated with aducanumab, vehicle ((b) (4) , 150 mM L-arginine HCl, and 0.05% [w/v] polysorbate 80), or negative control (saline). Positive controls (20% saponin for hemolysis, 20% Intralipid® for flocculation) performed as expected.

***In Vitro* Evaluation of the Influence of BIIB37 on Human Whole Blood Hemolysis and Plasma Flocculation**
(Biogen Idec Study P037-16-02; initiated November 24, 2016; GLP; QA)

Aducanumab (20 mg/mL) was evaluated for hemolytic potential and plasma flocculation by incubation with whole blood from 3 male and 3 female human donors for 1 hour at 37 °C. Hemolysis was evaluated by hematocrit, whole blood and plasma hemoglobin concentrations, plasma hemolytic index, and visual macroscopic hemolysis assessment. Flocculation was determined by plasma turbidity index and visual flocculation assessment.

There was no significant hemolysis or flocculation in any of the samples incubated with aducanumab, vehicle (16.2 mM L-histidine monohydrate, 3.8 mM L-histidine free base, 150 mM L-arginine HCl, 10 mM methionine, and 0.05% [w/v] polysorbate 80, pH 5.5), or negative control (saline). Positive controls (16% saponin for hemolysis, 20% Intralipid® for flocculation) performed as expected.

11 Integrated Summary and Safety Evaluation

Aducanumab-avwa is a human immunoglobulin gamma1 (IgG1) monoclonal antibody designed to bind to aggregated soluble and insoluble forms of amyloid beta (Aβ), resulting in the removal of amyloid plaques from the brain. The drug product is a sterile (b) (4) solution for IV infusion containing 100 mg/mL aducanumab and non-novel excipients. The proposed dosing regimen is IV infusion over approximately one hour every four weeks, titrating upward from 1 mg/kg (infusions 1 and 2) to 3 mg/kg (infusions 3 and 4) to 6 mg/kg (infusions 5 and 6) to 10 mg/kg thereafter. The proposed indication is for administration to patients with AD (b) (4)

Pharmacological studies of aducanumab (and murine analogs) evaluated species specificity, target selectivity, binding to Fc receptors, and pharmacodynamic activity. In vitro studies showed that the affinity of aducanumab for human Aβ42 monomers was approximately 40-fold greater than for mouse Aβ42 monomers. Multiple studies demonstrated that binding of aducanumab (and ^{ch}12F6A, a murine chimeric mAb retaining aducanumab's Fab variable antigen-binding regions) was much stronger to aggregated forms of Aβ40 and Aβ42 (plaques, fibrils, protofibrils, and oligomers) than to monomers. Epitope mapping showed that aducanumab binds to amino acids 3 to 7 in the Aβ peptide sequence. In vitro binding of aducanumab to human Fcγ receptors and to human complement C1q was similar to that of a human IgG1 control antibody.

In in vivo studies, weekly dosing of aged Tg2576 mice with intraperitoneal (IP) ^{ch}12F6A for 6 months resulted in significant reductions in cerebral insoluble Aβ40 and Aβ42, amyloid plaque number and area, and age-related impairment in a learning and memory task. These effects were not seen in animals dosed with ^{ch}12F6A Agly, a variant lacking the Fc effector function mediating phagocytosis of amyloid deposits by microglial cells. After a single administration of IP aducanumab to aged Tg2576 mice, aducanumab levels peaked in plasma and brain at 24 hours and 7 days postdose, respectively, and immunohistochemical analyses of brain sections showed binding to most parenchymal Aβ plaques and some of the vascular amyloid deposits.

Safety pharmacology parameters were assessed in the pivotal toxicology studies in monkey and Tg2576 mouse. There were no adverse effects on cardiovascular, CNS, or respiratory function as determined by ECG or clinical observations.

General toxicology studies included three 4-week studies of aducanumab in cynomolgus monkey and 13-week and 6-month studies of aducanumab and ^{ch}12F6A in aged Tg2576 mouse. There were no adverse effects in monkeys administered

aducanumab at 250 mg/kg/week SC, 300 mg/kg/week IV or SC, or (in the pivotal GLP study) 10, 100, or 300 mg/kg/week IV (the maximum feasible dose) for 4 weeks.

In the GLP 13-week study in aged Tg2576 mouse, administration of ^{ch}12F6A (0, 10, 70, or 500 mg/kg IV) weekly resulted in increased incidence and/or severity of meningeal vascular inflammation (minimal to moderate), thrombosis (minimal to mild), acute hemorrhage (mild), and perivascular lymphocytic infiltration (minimal to mild) in the brain in MD and HD animals; minimal meningeal vascular inflammation was present in MD and HD groups after 6 weeks of recovery. Similar effects were not seen in animals administered 500 mg/kg IV aducanumab, although slight increases were seen in brain microhemorrhages in F of this group and in the HDM ^{ch}12F6A group. Based on the meningeal vascular inflammation and acute hemorrhage in brain at the MD of 70 mg/kg/week, the NOAEL was the LD of 10 mg/kg/week IV ^{ch}12F6A.

There were no clear drug-related adverse effects in the GLP 6-month study of ^{ch}12F6A (0, 10, 40, or 250 mg/kg/week IV) or aducanumab (250 mg/kg/week IV) in aged Tg2576 mice. The inconsistent results between the 13-week and the 26-week studies may be related to the difference in the age of the animals at initiation of dosing (79-80 and 69-73 weeks old, respectively).

Genotoxicity studies of aducanumab were not conducted because antibodies are generally unable to interact with genetic material.

Carcinogenicity studies of aducanumab were not conducted because rat and mouse are not pharmacologically relevant species.

There were no adverse effects in fertility and early embryonic development, embryofetal development, and pre- and postnatal development studies conducted in rat at doses of 100, 300, and 1000 mg/kg/week IV aducanumab. However, the binding affinity of aducanumab to normal mouse Aβ (which has the same amino acid sequence as rat Aβ) was ~40 times lower than to human Aβ monomers. Therefore, rat does not appear to be a pharmacologically relevant species. Reproductive and developmental toxicity studies are generally not required by FDA for products indicated for the treatment of patients with dementia of the Alzheimer's type because of the age of the intended population.

Tissue cross-reactivity studies in Tg2576 mouse, monkey, and human tissues demonstrated specific binding of fluorescein-conjugated aducanumab to amyloid plaques in the cerebral cortex and hippocampus (Tg2576 mouse) and to blood vessel walls in the cerebral cortex (human and Tg2576 mouse), hippocampus and cerebellum (Tg2576 mouse), and fallopian tube (human). All other staining was cytoplasmic, and, therefore, clinically irrelevant based on the inability of aducanumab to enter cells.

In summary, drug-related adverse effects were limited to mild to moderate vascular inflammation, thrombosis, and hemorrhage in the brain of aged Tg2576 mice administered 70 or 500 mg/kg/week IV ^{ch}12F6A for 13 weeks, consistent with a slight exacerbation of the cerebral amyloid angiopathy expected in this mouse model. Drug-related increases Amyloid-Related Imaging Abnormalities (ARIA; vascular edema and

microhemorrhages) reported in clinical trials of aducanumab suggest the vascular findings may be relevant. There were no drug-related adverse effects in aged Tg2576 mice administered 10, 40, or 250 mg/kg/week IV ^{ch}12F6A for 6 months, or in cynomolgus monkeys administered 10, 100, or 300 mg/kg/week IV aducanumab for 4 weeks. In HD monkeys, plasma exposures (Day 22 $AUC_{0\text{-}168\ hrs}$) were 9-fold greater than the expected $AUC_{TAU,SS}$ in humans at the maximum proposed clinical dose of 10 mg/kg IV aducanumab. Adjusting for the differences in dosing frequency (QW in monkey vs. Q4W in human), estimated AUC exposure margins were ~35-fold.

Summary of Key Toxicities and Plasma Exposures in Pivotal Studies

Toxicity	Duration & Species	LOAEL or NOAEL	Mean AUC_{TAU}[#] (µg•hr/mL)	Exposure Margin Based on AUC*
No adverse effects	13-week Tg2576 mouse	10 mg/kg/wk ^{ch}12F6A (NOAEL)	11000 M 10300 F	-- --
Brain: mild mixed meningeal vascular inflammation and acute hemorrhage, minimal perivascular infiltration	13-week Tg2576 mouse	70 mg/kg/wk ^{ch}12F6A (LOAEL)	103000 M 74000 F	-- --
No adverse effects	6-month Tg2576 mouse	250 mg/kg/wk ^{ch}12F6A (NOAEL)	172000 M 114000 F	-- --
No adverse effects	4-week monkey	300 mg/kg/wk Aducanumab (NOAEL)	338000 M 358000 F	8.5x (34x) 9.0x (36x)

[#]Animal AUC_{TAU} values are means for the 168 hours (1 week) following the penultimate or final dose.
*Animal AUC_{TAU} divided by 39597 µg*hr/mL, the expected AUC_{TAU} at steady-state at the proposed maximum recommended human dose of 10 mg/kg IV every 4 weeks (x 4 to adjust for the 4-fold difference in dosing interval).

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/s/

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